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LUNAR-DAY VARIATIONS IN SPONTANEOUS ACTIVITY OF THE MONGOLIAN GERBIL[1]

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It is becoming increasingly apparent that organisms possess simultaneously a complex of biorhythms of varying frequencies. A number of small mammals including the white rat, white mouse and the golden hamster have been shown to possess at one and the same time both lunar-day and 24-hour cycles, while also displaying an overt circadian cycle (Brown, Shriner and Ralph, 1956; Brown and Terracini, 1959; Terracini and Brown, 1962; Brown, 1965; Brown and Park, 1967; Boyer, 1970). The 24.8-hour lunar-day cycle appears as the overt cycle in many organisms, particularly those inhabiting the intertidal zone of the ocean (Naylor, 1958; Enright, 1965; Hughes, 1972; Smith and Miller, 1973). In other organisms the lunar-day cycle is of very low amplitude and of a more obscure adaptive value (Terracini and Brown, 1962; Brown, 1965; Brown and Park, 1967; Klinowska, 1970, 1972; Brown and Chow, 1973b).

This paper will investigate whether activity in the Mongolian gerbil, *Meriones unguiculatus,* which has been shown to possess an obvious diurnal cycle (Stutz, 1972) and a more subtle cycle of a synodic monthly period (Stutz, 1973), possesses a lunar-day cycle as well. It is suspected that since a synodic monthly cycle results as a consequence of periodic beats between lunar and solar-day cycles, that a small-amplitude variation of a 24.8-hour period will exist. Should such a cycle be present, its presence would not be particularly adaptive for a desert-dwelling rodent, but more likely a consequence of living in an environment penetrated by subtle pervasive electromagnetic fields having a 24-hour and 50-minute cycle to which the animal is responsive.

MATERIALS AND METHODS

Gerbil spontaneous activity was monitored in small mammal actographs under two lighting regimes—3 foot candles of light from 6 A.M. to 6 P.M. (12–12 LD) and 3 foot candles of continuous light of 3 foot candles intensity (LL). Animals were kept in thermostatically controlled compartments (22 ± 1° C), with 2 animals per compartment. Food and water were present ad libidum and replenished every 5 to 8 days at random hours. Activity was monitored continuously by a 20-channel Esterline Angus Events Recorder and quantified by measuring the percentage of each hour that activity occurred. A scale from 0–10, at intervals of 1, was used, with a value of 10 indicating an animal to be active 100% of an hour.

One group of 6 gerbils was kept in 12–12 LD from May 1, 1968, through August 31, 1968. On September 1, 1968, two of these were removed and used in

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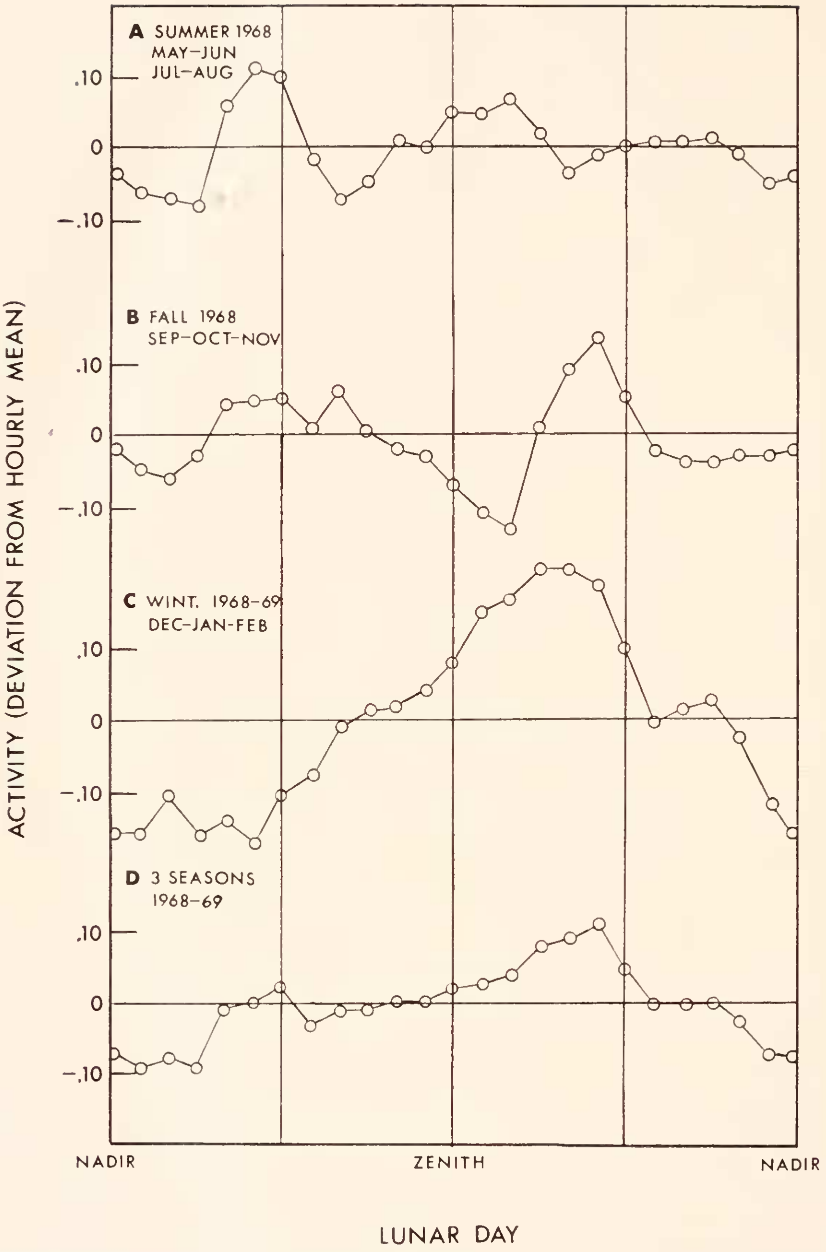

FIGURE 1. Curves A, B, and C represent lunar-day variations in spontaneous activity of gerbils in 12–12 LD for the summer, fall and winter seasons, respectively, in 1968–1969, expressed as deviation from each seasonal hourly mean. Curve D illustrates the mean three-season lunar-day cycle. Values given are three-hour sliding averages.

another experiment. The remaining 4 animals were kept in 12–12 LD through February 28, 1969. A second group of 4 animals was kept in LL from May 1, 1968, through March 15, 1969. Two additional animals which had been in activity cages in the open laboratory from May 1, 1968, were placed in LL on October 1, 1968, and remained in LL through March 15, 1969. Data from one of the animals in LL from May 1 were not used due to a malfunction of the recording apparatus.

An attempt was made to record animals in the two lighting regimes during approximately the same months, as comparisons between these two groups of data are most accurately made when data used cover similar time periods. Brown (1962) has reported an annual rhythm of a lunar-day frequency in fresh water planarians.

Results

12–12 LD

To demonstrate the existence of a lunar-day cycle, data corresponding to approximately the same hour of the lunar day were synchronized by moving successive days of data backwards under earlier days at the rate of 5 hours in 6 days. Since the overt solar-day cycle must be completely randomized, units of 29 days from each calendar month were used, for this permits the solar cycle to scan once per unit the 24 columns of data. This method of analyzing data for lunar-day rhythms has been described by Brown, Freeland and Ralph (1955).

Lunar-day cycles for each of three seasons were obtained by finding the mean amount of spontaneous activity per lunar hour for the season, assigning this seasonal hourly mean a value of zero, and plotting each individual average hourly value for the season as a deviation from this mean. Figure 1A shows the mean daily curve for 6 gerbils from May 1, 1968, to August 31, 1968. These 4 months will be considered the summer season. Figure 1B shows data gathered from 4 gerbils during fall, September 1, 1968, to November 30, 1968. Figure 1C represents similar data from 4 gerbils for the winter days extending from December 1, 1968, to February 28, 1969. The values for Figure 1D were derived in a manner similar to that used for Figures 1A–C, except that the 3-season hourly mean was given the value of zero and the 3-season mean values for each of the 24 hours were expressed as deviations from this mean. The lunar-day cycles in Figures 1A–D are plotted as 3-hour sliding averages.

Figure 1A, the lunar-day cycle for summer, is characterized as a tri-modal curve, with the major maximum at moonrise. A lesser maximum is present at lunar upper transit and extends through the two following hours. The third maximum, of rather low amplitude, occurs shortly after moonset. Minima are present over lunar lower transit, two hours after moonrise, and two hours before the setting of the moon. The range of this cycle is 10%, expressed as per cent of the hourly mean, which was given the value of 100%.

The lunar-day cycle in Figure 1B for the fall season is essentially a bimodal curve, with a maximum extending over moonrise and an even greater maximum, twice as high in amplitude, about one hour before moonset. Minima occur two hours after lunar upper transit and over lunar lower transit. The range of this cycle is 13%.

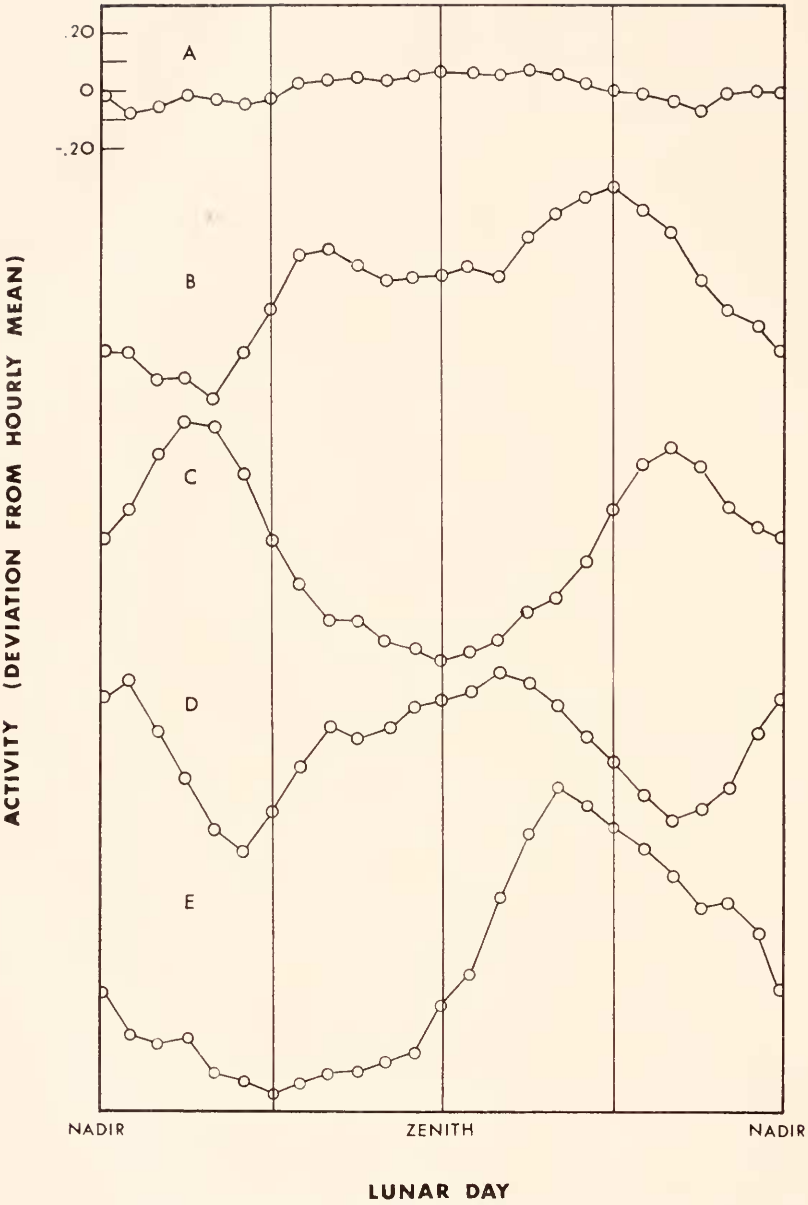

FIGURE 2. Curves A, B, C, D, and E show for gerbils A, B, C, D, and E, respectively, in LL the average lunar-day cycles expressed as deviation from the mean activity per lunar hour for the entire time period used for each animal. Values are three-hour sliding averages.

The lunar-day cycle for the winter season, shown in Figure 1C, is essentially a unimodal curve. The maximum occurs from one to three hours before the setting of the moon, and is almost twice as great in amplitude as maxima occurring in summer and fall. The minimum occurs at lower lunar transit and extends until one hour before moonrise. The range of the cycle for the winter season is 18%.

In Figure 1D, the lunar-day cycle for the combined three seasons is characterized as a bimodal curve. A conspicuous maximum occurs about one hour before moonset, with a subsidiary maximum present at the rising of the moon. A broad minimum is seen at lunar lower transit, while a lesser minimum occurs around lunar upper transit. The range of this three-season cycle is 10%.

When comparing the lunar-day components for the three seasons, it is interesting to note that the form of the summer and fall cycles parallel one another from lunar hours zero through seven, and are very nearly the mirror-image of each other for the remaining hours of the lunar day. The greatest amount of spontaneous activity during the winter season occurs at the same hours of day as it does during the fall. For winter the only minimum, and for summer the lowest minimum occurs over lower lunar transit. The cycle for the fall season also exhibits a broad minimum at this time. The tendency for lunar inversion seems to be great, and maxima and minima appear to be phase-locked to the lunar hours at or close to moonrise, moonset, lunar upper and lower transits.

Constant light (*LL*)

Data gathered under LL were examined for the existence of a lunar-day cycle in an attempt to verify further the presence of a lunar-day component in the gerbil. Methods of analyzing the data for a lunar-day cycle were similar to those used in the previous section, but in this case both the solar-day cycle and the overt circadian cycle had to be randomized, necessitating period lengths which were simple multiples both of 29.5 days and the period necessary for the circadian cycle to scan the day. Therefore, data for each gerbil were treated as an individual unit of approximately 179 days, and data could not be analyzed for the presence of a seasonal variation.

Figures 2A-E show, for gerbils A, B, C, D, and E, respectively, the lunar-day variation in activity remaining after randomization of overt circadian and solar-day cycles. The following are the period of time (days) used for each animal in this examination: *Gerbil A* (in LL fom May 1, 1968, to March 15, 1969): July 1–December 31, 184 days; *Gerbil B* (in LL from October 1, 1968, to March 15, 1969): October 17–March 15, 149 days; *Gerbil C* (in LL from May 1, 1968, to March 15, 1969): August 1–February 16, 200 days; *Gerbil D* (in LL from October 1, 1968, to March 15, 1969): October 1–February 28, 151 days; *Gerbil E* (in LL from May 1, 1968, to March 15, 1969): August 1–February 28, 212 days. Figure 3 shows the mean cycle for all five gerbils. All values in Figures 2 and 3 are 3-hour sliding averages.

Figures 2A and 2B are very similar, in that times of greatest activity occur when the moon is above the horizon and least activity occurs when the moon is below the horizon. Figure 2A is essentially a unimodal curve of extremely low amplitude, with its maximum at lunar upper transit and minimum around lunar lower transit. Figure 2B may be described further as a bimodal curve with the major minimum around the time of lunar lower transit, and a second minimum at lunar upper transit. Maxima occur around moonrise and moonset. In Figure 2C, a bimodal curve, gerbil activity is greatest essentially when the moon is below the horizon, with maxima occurring close to moonrise and moonset. The least amount

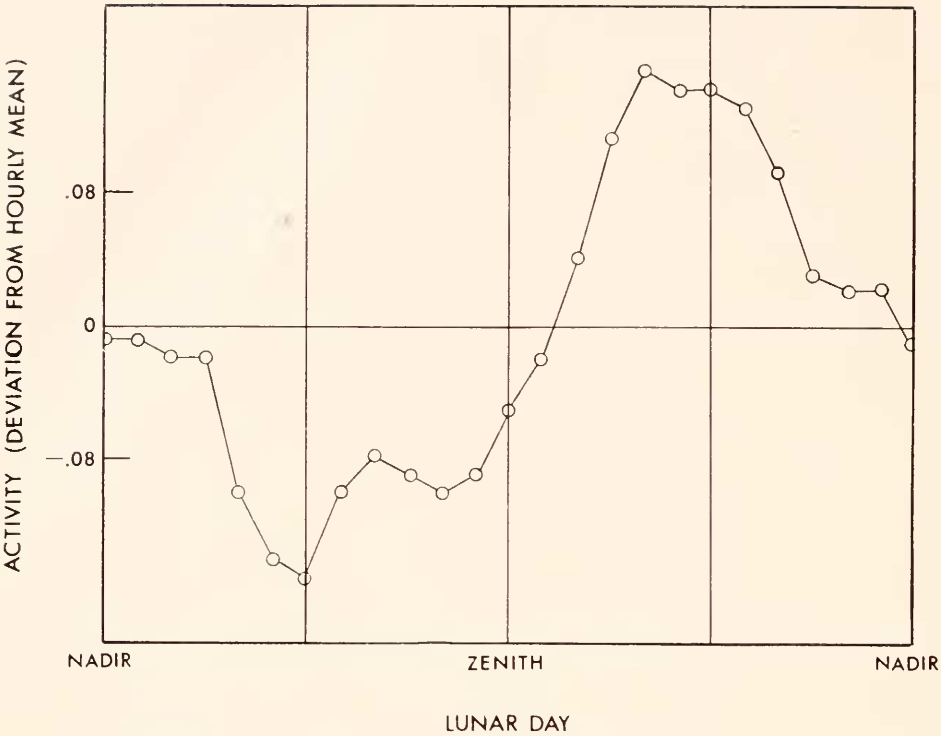

FIGURE 3. Mean lunar-day cycle for all 5 gerbils—A, B, C, D, and E—in LL. Values are three-hour sliding means.

of activity is during the lunar day, falling to a low at lunar upper transit, with a secondary minimum at lunar lower transit. Figures 2C and 2D are almost perfect mirror images of one another, with gerbil D showing maximum activity when the moon is above the horizon, rising to a peak close to upper transit. A second peak of activity is present at lunar lower transit. Minima occur around times of moonrise and moonset. Figure 2E, a unimodal curve, shows maximum gerbil activity two hours before the setting of the moon and minmum activity at moonrise.

The mean lunar-day cycles for all gerbils seem to have times of maxima and minima locked to the approximate hours of moonrise, moonset, lunar upper and lower transits. There is also an apparent tendency for the cycles to undergo major inversions of one another, and it appears that the cycles are all interrelated with one another in terms of degrees of inversion. These phenomena were also found to be true for those lunar-day activity cycles disclosed in gerbils in 12–12 LD.

Figure 3, the mean lunar-day cycle for gerbils A–E, shows a broad maximum extending over the time of moonset. A secondary, and very low amplitude maximum, is present two hours after moonrise. The major minimum of this cycle is at the time of the rising of the moon.

Expressed as percentages of the mean hourly average, the ranges of the lunar-day cycles in Figure 2 are 11%, 39%, 46%, 29% and 47% for gerbils A, B, C, D, and E, respectively. The range of the mean cycle, Figure 3, for all 5 gerbils is 16%. This range is substantially reduced due to the frequency of cycle inversion found among the 5 gerbils. None of these ranges is insignificant. In fact, the range of 4 out of 5 of the gerbil cycles are quite large compared with those found for lunar-day cycles in other organisms.

Discussion

When appropriate care was used in randomizing the underlying solar and overt circadian rhythms in those gerbils held in LL, a rather large-amplitude average rhythm of a lunar-day frequency was revealed, with maxima close to times of rising and setting of the moon and a conspicuous minimum at lower lunar transit. The lunar-day cycle disclosed in those animals held in 12–12 LD was very similar in form and phase relationship to that of the gerbils in LL.

The phenomenon of inversion of the lunar-day cycle over all or parts of the lunar day was found to exist from season to season in those gerbils in 12–12 LD and also from animal to animal in those gerbils studied individually in LL. Studies of rhythmicity in a variety of organisms have shown that similar periodic inversions of phase, with maintenance of otherwise the same frequency, are common among living things.

Just as underlying solar-day rhythms in the gerbil and a number of other forms appear to be related to one another in cycle form, so also does a similar relationship exist for lunar-day fluctuations. The mean lunar-day cyclic pattern in Figure 1D very closely resembles that of the potato (Brown, 1960), *Fucus* (Brown, Freeland and Ralph, 1955), earthworm (Ralph, 1957), salamander (Brown, Webb, Bennett and Sandeen, 1955), white mouse (Terracini and Brown, 1962), and mealworm (Campbell, 1964). The cycle for the summer season, May through August, is also very similar to that of the golden hamster for the summer lunar month of July–August 1967 (Klinowska, 1972). This similarity, or an inversion of it, throughout all or part of the lunar day, in cyclic pattern between the gerbil and a variety of other species strongly supports the hypothesis that all organisms were influenced by subtle factors of a 24.8-hour frequency penetrating laboratory "constant" conditions.

Recent studies indicate that weak electrostatic fields, gamma radiation and geomagentism, which penetrate easily through buildings and possess the necessary period lengths, may be the timing mechanisms for biological clocks (Brown, 1972; Brown and Chow, 1973a). In addition to their potential role as biological clocks, this family of weak fields has been shown to play other vital roles in the daily lives of organisms (Lindauer and Martin, 1968; Keeton, 1971; Brown, 1971; Wiltschko and Wiltschko, 1972; Rommel and McCleave, 1972; and many others).

Evidence that gerbils respond to the weak horizontal magnetic field or its correlate was shown by Stutz (1971). Unexpected variation in gerbil spontaneous activity from 3–6 P.M. was shown to be correlated (an average of $r = +0.45$; $P = 0.01$; $N = 30$, for each of 6 consecutive months) with the amount of change in horizontal magnetic intensity for the concurrent time period. Significant correlations between the two parameters were not found during any other time periods throughout the day.

The difficulty in imaging a function for a lunar-day component in the terrestrial, desert-dwelling gerbil lends further credibility to the conclusion that the lunar-day periodism in this animal is merely a consequence of existence in a periodic geophysical atmosphere. Brown (1968) hypothesized that the lunar adaptive functions are synchronized to an underlying phase-stable period of a lunar-day frequency in such a manner as to consist of a recycling "tape" which initiates a period of phase-labile activity firmly "coded" to a certain location on the phase-stable tape. In accordance with this hypothesis, it is felt that the gerbil lunar-day phase-stable tape would remain relatively uncoded. Lunar-day periodisms in hamsters (Brown, 1965) and a rat (Brown and Terracini, 1959) have been reported to gain timing control and to hold for an extended time period a behavioral pattern normally a solar-day adaptation. These findings do suggest that the lunar-day phase-stable tape of a land-dwelling species could, however, potentially be coded with a complex pattern of behavior.

SUMMARY

1. Spontaneous activity of male gerbils, *Meriones unguiculatus*, kept in actographs was recorded continuously from May 1, 1968, through March 15, 1969, in Evanston, Illinois. Gerbil activity was studied under two lighting conditions: constant illumination (LL) and 12–12 LD.

2. For those gerbils kept in 12–12 LD a lunar-day periodism of spontaneous activity was disclosed for each of three seasons(summer, fall, and winter). The mean three-season cycle in 12–12 LD exhibited maxima around times of rising and setting of the moon and minima at lunar lower and upper transits.

3. For those 5 animals in LL a lunar-day cycle of activity was reported for each animal over a period averaging 179 days. When the lunar-day cycles for each of the 5 gerbils in LL were averaged together, a mean periodism with cycle form and phase relationship very similar to that of the three-season cycle for gerbils in 12–12 LD was found. The range of this cycle was 16%.

4. All lunar-day cycles studied had maxima and minima locked to the approximate hours of moonrise, moonset, lunar upper and lower transits. There was a strong tendency for lunar inversion, but the cycles appeared to be interrelated with one another in degrees of inversion.

5. These results give support to the hypothesis that the gerbils are responsive to weak geoelectromagnetic fields with period lengths of 24.8 hours from which they are not shielded in the laboratory.

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